REMARKS

Claims 1-42 are pending in the application. Claims 25-42 have been canceled as being drawn to a non-elected invention. Claims 1-24 have been examined and stand rejected. Claims 1, 6, 8, 9, 12, and 21 have been amended. Claims 5 and 7 have been canceled. No new matter has been introduced. Reconsideration and allowance of Claims 1-4, 6, and 8-24 is respectfully requested.

Response to Restriction Requirement

In response to the Examiner's requirement for election of a single invention between Group I (Claims 1-24), drawn to a method for amplifying microRNA, and Group II (Claims 25-42), drawn to a kit comprising a primer set or individual primers, on June 17, 2009, applicant orally elected to prosecute the invention of Group I (Claims 1-24), without traverse.

The Objection to the Specification

The specification has been amended to remove the embedded hyperlinks on page 6, line 27, and on page 13, line 18, as requested by the Examiner. Removal of the objection to the specification is respectfully requested.

The Rejection of Claims 1, 3, 10, 15, 18, and 20 Under 35 U.S.C. § 102(b) as Being Anticipated by Lau et al., *Science 294*:858-862 (2001), as Evidenced by Lau Supplemental Information

Claims 1, 3, 10, 15, 18, and 20 stand rejected under 35 U.S.C. § 102(b) as being anticipated by Lau et al., *Science 294*:858-862 (2001), as evidenced by Lau Supplemental Information. Applicant respectfully traverses this ground of rejection for at least the following reasons.

While not acquiescing with the Examiner's position, but in order to clarify the invention, Claim 1 has been amended to incorporate the limitation of Claim 5, now canceled.

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Claim 1, at step (a) as amended, recites "(a) producing a first DNA molecule that is complementary to a target microRNA molecule using primer extension with an extension primer comprising a first portion selected to hybridize to a portion of the target microRNA molecule and a second portion that hybridizes to the complement of a universal forward primer . . . ."

The Examiner admits that Lau et al. does not teach that the extension primer comprises a first portion that hybridizes to the target microRNA. Office Action at page 11. Therefore, for at least this reason, it is demonstrated that Claim 1 is not anticipated by Lau et al. Claims 3, 10, 15, 18, and 20 depend from Claim 1 and are therefore also not anticipated by Lau et al. for at least the reason described in connection with Claim 1. Removal of this ground of rejection is respectfully requested.

The Rejection of Claims 2, 13-14, 16-17, and 22 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lau et al. (2001), in View of Braasch et al., *Chemistry & Biology*, pages 1-7 (2001)

Claims 2, 13-14, 16-17, and 22 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lau et al. (2001), in view of Braasch et al., *Chemistry & Biology*, pages 1-7 (2001). Applicant respectfully traverses this ground of rejection for the following reasons.

Claims 2, 13, 14, and 16-17 depend directly or indirectly from Claim 1. Claim 1 has been amended to incorporate the limitations of Claim 5 as described *supra*. As described above, the Examiner admits that Lau et al. does not teach that the extension primer comprises a first portion that hybridizes to the target microRNA. Office Action at page 11. Moreover, Lau does not remotely teach or suggest the claimed invention. In sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, the teachings of Lau is directed to the non-specific amplification of all microRNAs in a sample through the ligation of adaptor linkers for the purpose of cloning previously unidentified microRNA species.

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Claim 22 depends from Claim 21. Independent Claim 21 has been amended to recite at step (1) "producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension with an extension primer comprising a first portion selected to hybridize to a portion of the target microRNA molecule and a second portion that hybridizes to the complement of a universal forward primer . . . ." As described above, the Examiner admits that Lau et al. does not teach that the extension primer comprises a first portion that hybridizes to the target microRNA.

It is therefore demonstrated that a *prima facie* case of obviousness has not been established because Lau and Braasch taken together or separately, fail to teach every limitation of Claims 1 and 21 as amended. Accordingly, Claims 2, 13-14, 16-17, and 22, are believed to be patentable over Lau et al. (2001), in view of Braasch et al., *Chemistry & Biology*, pages 1-7 (2001). Removal of this ground of rejection is respectfully requested.

The Rejection of Claims 5-7, 12, 19, and 21 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lau et al. (2001) in View of U.S. Patent Publication No. 2003/0186288 (Spivack et al.)

Claims 5-7, 12, 19, and 21 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lau et al. (2001) in view of U.S. Patent Publication No. 2003/0186288 (Spivack et al.).

The Examiner characterizes Lau et al. as disclosing a method for measuring the amount of a target microRNA in a sample comprising the steps of:

> (1) producing a first DNA molecule complementary to the target microRNA molecule in the sample using primer extension (p. 862, col 1, 23, where the size fractionated RNAs are ligated with 3' adaptor using T4 RNA ligase, followed by ligating a 5' adaptor and the ligation products were reverse transcribed resulting in cDNA, see p. 4 supplemental information); (2) amplifying the first DNA molecule to produce amplified

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DNA molecules using a universal forward and reverse primer (p.862, col.1, 23, where the cDNA was amplified using primers corresponding to the adaptor sequences, as evidenced by p.4 of supplemental information where cDNA was amplified using the original RT primer and a primer complementary to the 5' adaptor). [Office Action at pages 10-11.]

The Examiner further characterizes Lau et al. as disclosing an extension primer comprising a second portion, in which the RT primer is complementary to the adaptor.

With regard to Claims 5-8 and 12, the Examiner admits that Lau et al. does not teach that the extension primer comprises a first portion that hybridizes to the target microRNA. With regard to Claim 21, the Examiner admits that Lau et al. does not explicitly teach measuring the amount of amplified DNA molecules.

The Examiner characterizes Spivack as teaching an extension primer that comprises a first portion that hybridizes to the target microRNA with reference to Figures 6 and 7, where the cDNA extension primer comprises a tag sequence with a unique sequence. The Examiner notes that Spivack teaches a novel PCR strategy that takes advantage of the poly-A tail of processed mRNA. With regard to Claim 21, the Examiner further characterizes Spivack as teaching measuring the amount of amplified DNA molecules, wherein the amplification products are subjected to agarose or polyacrylamide gel electrophoresis, labeled with a fluorescent moiety, or measured using fluorescence-based quantitative PCR. The Examiner further notes that Spivack teaches "the novel Universal RT primer used for reverse transcription has a 3' three-base anchor that allows the primer to be positioned on the last 3 bases of the transcript specific sequence and covers all possible combinations of the coding 3' end of the mRNA transcript."

The Examiner contends that it would have been obvious to one of ordinary skill in the art at the time the invention was made to have adjusted the teachings of Lau to include both a tag

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sequence and a portion specific to the target as taught by Spivack to arrive at the claimed invention with a reasonable expectation for success.

Applicant respectfully traverses this ground of rejection for the following reasons.

As an initial matter, without acquiescing to the Examiner's position, but in order to facilitate prosecution, independent Claim 1, from which Claims 6, 12, and 19 depend, and independent Claim 21 have been amended to recite "using primer extension with an extension primer comprising a first portion selected to hybridize to a portion of the target microRNA molecule and a second portion that hybridizes to the complement of a universal forward primer . . . ." Claims 5 and 7 have been canceled.

The cited references, either alone or in combination, fail to render the claimed invention unpatentable. KSR confirmed that the Grahm Factor Analysis should be used in determining whether a claimed invention is obvious under Section 103(a). *KSR Int'l Co. v. Teleflex Inc.*, 127 S.Ct. 1727, 1739 (2007). This analysis includes assessing the rejected claims, the scope and content of the cited art, and the differences between the rejected claims and the cited art. Id. at 1734. As will be shown, a *prima facie* case of obviousness has not been established because (1) the references taken together or separately, fail to teach every limitation of the claimed invention; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because Spivack teaches directly away from the combination; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention.

1. The Differences between the Rejected Claims, as amended, and the Cited Art

Lau et al.

Lau et al. does not teach or suggest the use of an extension primer comprising a first portion that hybridizes to the target microRNA. In contrast to the claimed invention, Lau et al. is

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directed to the cloning of endogenous C. elegans miRNAs and the discovery of 55 previously unknown miRNAs in C. elegans. Abstract. Lau describes the construction of an amplified small RNA library by first ligating 3' RNA adaptor oligonucleotides to a pool of gel-purified 18-26nt small RNAs from mixed-stage worms with T4 RNA ligase, gel purifying the ligated RNA, then ligating to a 5' adaptor oligonucleotide in a second T4 RNA ligase reaction, gel purifying the products from the second ligation followed by reverse transcription and PCR amplification of the linker-ligated products using DNA oligos *corresponding to the adaptor sequences*. The PCR products were submitted for sequencing. See Footnote 23 and Lau supplementary materials.

Moreover, Lau et al. does not remotely teach or suggest that the above described cloning method could be used for measuring the amount of a target microRNA in a sample. Rather, Lau et al. teaches the use of Northern blots for measuring microRNA expression. See pages 859 and 861, and FIGURE 3.

Spivack et al.

Contrary to the Examiner's contention, Spivack et al. does not teach or suggest the use of an extension primer comprising a first portion selected to hybridize to a portion of the target microRNA molecule, as claimed. Rather, Spivack et al. discloses the use of an RT-PCR strategy that detects mRNA with a primer that hybridizes to the polyA tail of the mRNA. See, for example, paragraph [0103] of Spivack, which states, "The methods of the present invention can be used to analyze gene expression form any material in which genes are expressed to generate mRNA molecules having poly-A tails." As described in paragraph [0057] of Spivack, the RT primers used to perform reverse transcription comprise (a) a 3' anchor sequence; (b) a poly T midsection to anneal to the polyA tail of the mRNA; and (c) a 5' tag. Spivack et al. does not disclose or suggest a method using an extension primer that specifically hybridizes to a target

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microRNA as claimed, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail.

2. The Differences between the Rejected Claims and the Cited Art Are Not Obvious Differences

There is no apparent reason to combine Lau and Spivack as asserted by the Examiner. Moreover, even if the teachings of Lau and Spivack were to be combined, such a combination would not result in the claimed invention, where an extension primer that specifically hybridizes to a target microRNA is used to amplify microRNA. Further, the combination would not result in the claimed invention because there is no ability to quantitatively measure a particular target microRNA. In sharp contrast to the claimed invention, which is directed to methods for quantitating specific target microRNAs, the teachings of Lau and Spivack are both directed to the non-specific amplification of either all microRNAs in a sample through the ligation of adaptor linkers (Lau), or through the use of oligo-dT priming of all polyA+ mRNA (Spivack). Thus, no combination of the cited references renders the claimed invention obvious. See MPEP § 2143.02 and KSR, 127 S.Ct. at 1741.

In addition to this finding, applicant respectfully disagrees with the motivation to combine these references in the manner asserted by the Examiner. In the context of an obviousness rejection, the Supreme Court explained the importance of "identify[ing] a reason" why a skilled artisan would be prompted to arrive at the presently claimed invention. KSR, 127 S. Ct. at 1741. The Court noted that there should be an "explicit" analysis regarding "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." Id. Because the combination of references fails to teach every limitation of the present claims, there can be no "apparent reason" to combine the references to arrive at the presently claimed invention.

As noted above, contrary to the Examiner's contention, Spivack describes the use of an RT-PCR strategy that detects mRNA with a primer that hybridizes to the polyA tail of the mRNA. Spivack does not teach or suggest a method comprising a primer that specifically hybridizes to a target microRNA, because, as known by those of skill in the art, a mature microRNA does not contain a polyA tail. Therefore, there is no apparent reason why one of skill in the art would modify the teachings of Lau et al. to include a region to hybridize to a polyA tail when the target microRNA is known to lack a polyA tail.

In fact, Spivack actually teaches directly away from the claimed invention with the teaching that the use of target specific primers are undesirable due to the requirement of very specific annealing conditions (page 2), the fact that new reverse transcription primers would be required for each transcript target to be analyzed, and the inefficient use of RNA in target-specific analysis.

As noted in the present application, prior to the present invention, the amplification of small RNA molecules was difficult to those skilled in the art. As stated in the instant specification,

> Short RNA molecules are difficult to quantitate. For example, with respect to the use of PCR to amplify and measure the small RNA molecules, most PCR primers are longer than the small RNA molecules, and so it is difficult to design a primer that has significant overlap with a small RNA molecule, and that selectively hybridizes to the small RNA molecule at the temperatures used for primer extension and PCR amplification reactions.

See page 2, lines 20-25, of the specification published as International Publication No. WO 2006/081284.

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Therefore, it is demonstrated that a *prima facie* case of obviousness has not been established because (1) Lau and Spivack taken together or separately, fail to teach every limitation of the claimed invention; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because Spivack teaches directly away from the combination; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention. Accordingly, removal of this ground of rejection is respectfully requested.

The Rejection of Claim 8 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lau et al. (2001) in View of Spivack et al.

Claim 8, which depends from Claim 1 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Lau et al. (2001) in view of Spivack et al. Applicant respectfully traverses this ground of rejection for at least the following reasons.

As discussed above with respect to Claim 1, Lau et al. and Spivack et al., either separately or together, fail to teach or suggest even element of the claimed invention. Further, no motivation exists to combine these references and there is no reasonable expectation of success to achieve the claimed invention with respect to such a hypothetical combination. Thus, as with Claim 1, this dependent claim is also not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

The Rejection of Claims 9 and 11 Under 35 U.S.C. § 103(a) as Being Unpatentable Over Lau et al. (2001) in View of Spivack et al., Further in View of Crollius et al., *Nature Genetics 25*(2):235-238 (2000), in Further View of Buck et al., *Biotechniques 27*:528-536 (1999)

Claims 9 and 11, which depend from Claim 1, stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Lau et al. (2001) in view of Spivack et al. in further view of Crollius et al., *Nature Genetics* *25*(2):235-238 (2000), in further view of Buck et al.,

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Biotechniques 27:528-536 (1999). Applicant respectfully traverses this ground of rejection for at least the following reasons.

As discussed above with respect to Claim 1, Lau et al. and Spivack et al., either separately or together, fail to teach or suggest even element of the claimed invention. Further, no motivation exists to combine these references and there is no reasonable expectation of success to achieve the claimed invention with respect to such a hypothetical combination. The teachings of Crollius et al. and Buck et al. fail to cure the deficiencies of Lau and Spivack in this regard.

The Examiner cites Crollius as disclosing an oligonucleotide comprising SEQ ID NO:1 or 13. It is noted that there is no teaching or remote suggestion in Crollius with regard to a method using an extension primer that specifically hybridizes to a target microRNA for amplifying the target microRNA. Rather, Crollius et al. is directed to the use of pufferfish Tetraodon nigroviridis to detect conserved sequences in the human genome with low background in order to estimate the number of genes in the human genome (abstract). The Examiner cites Buck et al. as disclosing evidence of the equivalence of primers. It is noted that there is no teaching or remote suggestion in Buck et al. with regard to the use of an extension primer that specifically hybridizes to a target microRNA.

Therefore, it is demonstrated that a *prima facie* case of obviousness has not been established because (1) Lau, Spivack, Crollius, and Buck et al. taken together or separately, fail to teach every limitation of Claim 1, as amended; (2) there is no motivation or expectation of success to combine the references to arrive at the claimed invention because Spivack teaches directly away from the combination; and (3) the hypothetical combination proposed by the Examiner does not result in the claimed invention. Thus, as with Claim 1, these dependent claims are also not obvious over the cited art. Applicant respectfully requests withdrawal of this ground of rejection.

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Conclusion

In view of the foregoing amendments and remarks, it is believed that all the pending claims are in condition for allowance. Reconsideration and favorable action are requested. If any issues remain that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicant's attorney at 206.695.1655.

Respectfully submitted,

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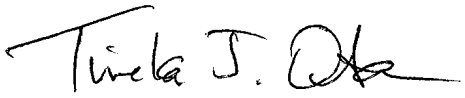

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